

Claims

1. A method for regulating the expression of a gene of interest in a host cell that comprises a CodY-like protein comprising providing said cell with a gene of interest in operable linkage with a promoter and at least one CodY target sequence.
- 5 2. A method according to claim 1, wherein said promoter and/or said CodY target sequence is heterologous with regard to said gene of interest.
3. A method according to claim 1 or 2, wherein said CodY target sequence is heterologous with regard to said promoter.
4. A method according to any one of claims 1 to 3, wherein said gene of
10 interest is a gene from a gram-positive bacterium.
5. A method according to any one of claims 1 to 4, wherein said gene of interest encodes a protease or a peptidase or an anti-microbial peptide or a vitamin.
6. A method according to any one of claims 1 to 5, wherein said CodY
15 target sequence comprises a sequence as depicted in Figure 6A or a functional equivalent and/or a functional fragment thereof.
7. A method according to any one of claims 1-6, wherein said CodY target sequence comprises a sequence as depicted in Figure 6B or a functional equivalent and/or a functional fragment thereof.
- 20 8. A method according to any one of claims 1-7, wherein said CodY target sequence comprises a sequence as depicted in Table 4, Table 4A, Table 5, Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a functional fragment thereof.
9. A method according to any one of claims 1-8, wherein said CodY target
25 sequence comprises an ATGTTCA sequence and an inversely repeated ATGTTCA sequence.

10. A method according to claim 9, wherein said nucleic acid sequence comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
11. A method according to claim 9 or 10, wherein said nucleic acid sequence
5 comprises the sequence ATGTTTCAGAAAATTCATGAACAT.
12. A method according to any one of claims 1 to 11, further comprising influencing the binding between said CodY-like protein and said at least one CodY target sequence.
13. A method according to claim 12, wherein said binding is regulated by
10 subjecting said cell to a change in a growth condition.
14. A method according to claim 12 or 13, wherein said binding is regulated by subjecting said cell to a growth limiting condition.
15. A method according to claim 14, wherein said growth limiting condition is a limited availability of a nitrogen source.
- 15 16. A method according to any one of claims 1 to 15, wherein said host cell is a cell from a (dairy) food production species.
17. A method according to claim 16, wherein said species is selected from a *Lactococcus* or *Lactobacillus* or *Streptococcus* or *Leuconostoc* or *Pediococcus* or *Bifidobacterium* or *Carnobacterium* or *Propionibacterium*.
- 20 18. A method according to any one of claims 1 to 17, wherein said host cell is provided with a nucleic acid encoding a CodY-like protein.
19. An isolated or recombinant nucleic acid that comprises at least one CodY target sequence or a functional fragment and/or a functional equivalent thereof.
- 25 20. A nucleic acid according to claim 19, further comprising a promoter in operable linkage with a gene of interest.
21. A nucleic acid according to claim 19 or 20 further comprising a gene encoding a CodY-like protein.

22. A nucleic acid according to claim 20 or 21, wherein said promoter and/or said at least one CodY target sequence is heterologous with regard to said gene of interest.
23. A nucleic acid according to any one of claims 20 to 22, wherein said
5 CodY target sequence is heterologous with regard to said promoter.
24. A nucleic acid according to any one of claims 20 to 23, wherein said gene of interest is a gene from a gram-positive bacterium.
25. A nucleic acid according to any one of claims 20 to 24, wherein said gene of interest encodes a protease or a peptidase or an anti-microbial peptide or a
10 vitamin.
26. A nucleic acid according to any one of claims 19 to 25, wherein said CodY target sequence comprises a sequence as depicted in Figure 6A or a functional equivalent and/or a functional fragment thereof.
27. A nucleic acid according to any one of claims 19 to 26, wherein said
15 CodY target sequence comprises a sequence as depicted in Figure 6B or a functional equivalent and/or a functional fragment thereof.
28. A nucleic acid according to any one of claims 19 to 27, wherein said CodY target sequence comprises a sequence as depicted in Table 4, Table 4A, Table 5, Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a
20 functional fragment thereof.
29. A nucleic acid according to any one of claims 19 to 28, wherein said CodY target sequence comprises an ATGTTCA sequence and an inversely repeated ATGTTCA sequence.
30. A nucleic acid according to claim 29, wherein said nucleic acid sequence
25 comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
31. A nucleic acid according to claim 29 or 30, wherein said nucleic acid sequence comprises the sequence ATGTTTCAGAAAATTCATGAACAT.
32. A vector comprising a nucleic acid according to any one of claims 19 to
30 31.

33. A gene delivery vehicle comprising a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32.
34. A host cell comprising a nucleic acid according to any one of claims 19 to 31, a vector according to claim 32 or a gene delivery vehicle according to claim 5 33.
35. A host cell according to 34 which is a cell from a (dairy) food production species.
36. A host cell according to claim 34 or 35, wherein said species is selected from a *Lactococcus* or *Lactobacillus* or *Streptococcus* or *Leuconostoc* or 10 *Pediococcus* or *Bifidobacterium* or *Carnobacterium* or *Propionibacterium*.
37. Use of at least one CodY target sequence for regulating the expression of a gene of interest.
38. Use according to claim 37, wherein said target sequence comprises a sequence as depicted in Figure 6A, Figure 6B, Table 4, Table 4A, Table 5, 15 Table 6, Table 7 and/or Table 8, or a functional equivalent and/or a functional fragment thereof.
39. Use according to claim 37 or 38, wherein said CodY target sequence comprises an ATGTTCA sequence and an inversely repeated ATGTTCA sequence.
- 20 40. Use according to any one of claims 37 to 39, wherein said nucleic acid sequence comprises a spacing of about 9 base pairs between said ATGTTCA sequence and said inversely repeated ATGTTCA sequence.
41. Use according to any one of claims 37 to 40, wherein said nucleic acid sequence comprises the sequence ATGTTTCAGAAAATTCATGAACAT.
- 25 42. A method for producing a (dairy) food product comprising a step wherein a nucleic acid according to any one of claims 19 to 31, a vector according to claim 32, a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 is used.
43. A method according to claim 42, wherein said dairy product is a cheese 30 or a fermented milk product.

44. A cheese or a fermented milk product obtainable by a method according to claim 42 or 43.
45. A method for at least in part preventing the formation of off-flavours during a process for the production of a (dairy) food product, comprising
- 5 providing at least one CodY target sequence upstream of a gene which product is, directly or indirectly, involved in the formation of off-flavours.
46. Use of a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32 or a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 for increasing the expression of a
- 10 gene of interest in a stationary phase culture or equivalents of said culture.
47. Use according to claim 46, wherein said gene of interest comprises a gene encoding an antimicrobial substance, such as a bacteriocin.
48. Use according to claim 46, wherein said gene of interest comprises a gene encoding a flavour compound, vitamin, or a proteinaceous molecule
- 15 involved in cell lysis.
49. Use of a nucleic acid according to any one of claims 19 to 31 or a vector according to claim 32 or a gene delivery vehicle according to claim 33 or a host cell according to any one of claims 34 to 36 for decreasing the expression of a gene in a stationary phase culture or equivalents of said culture.
- 20 50. Use according to claim 49, wherein an antisense nucleic acid sequence of an undesired gene is provided in operable linkage with a promoter and at least one CodY target sequence.
51. Use according to claim 49 or 50, wherein said gene is involved with acidification.